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CHROMOSOME KARYOTYPES OF THREE BIVALVES: THE OYSTERS, *ISOGNOMON ALATUS* AND *PINCTADA IMBRICATA*, AND THE BAY SCALLOP, *ARGOPECTEN IRRADIANS IRRADIANS*[1]

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Since Menzel and Menzel (1965) reported on the chromosomes of two species of clams, several workers have observed the chromosomes of bivalves (pelecypod molluscs) using squash or air-drying techniques (Ahmed and Sparks, 1967, 1970; Longwell, Stiles and Smith, 1967; Menzel, 1968; Patterson, 1970; Ieyama and Inaba, 1974; Ieyama, 1977). However, there are still only a few species of bivalves for which chromosome numbers have been established, as compared with the greater numbers of gastropod molluscs with known chromosome karyotypes.

This paper presents the chromosome number and gross morphology of three species of Bivalvia not previously reported: *Isognomon alatus,* the flat tree oyster; *Pinctada imbricata,* the Atlantic pearl oyster; and *Argopecten irradians irradians,* the commercial bay scallop.

Materials and Methods

Specimens of *Isognomon alatus* were collected in June, 1976, from Biscayne Bay, Miami, Florida. Specimens of *Pinctada imbricata* were collected in February, 1977, near Pompano Beach, Florida. Cultured specimens of *Argopecten irradians irradians* were obtained from a commercial hatchery on Long Island, New York.

Eggs and sperm were obtained by stripping the gonads of *I. alatus* (10 animals). Spawning was induced thermally in *P. imbricata* (14 animals) and in *A. i. irradians* (25 animals). *A. i. irradians* is hermaphroditic and usually spawns both eggs and sperm almost simultaneously. When unfertilized eggs were needed, these animals were induced to spawn in sea water containing 0.02% EDTA (ethylene-diaminetetraacetic acid) which, by chelating calcium out of the sea water, must prevent the acrosome reaction of the sperm essential for fertilization. Self-fertilization was effectively prevented by the EDTA. EDTA-exposed eggs were washed with fresh sea water just after spawning, and no chromosome damage was observed in them. Following fertilization, eggs and embryos were fixed at intervals in Carnoy fixative (3:1). Chromosome preparations were made by squashing the eggs or embryos in 1 to 2% aceto-orcein.

To obtain colchicine metaphase in some cleaving eggs, fertilized eggs were held in sea water containing 0.02% colchicine for 15 to 30 minutes. *P. imbricata* eggs were not treated with colchicine.

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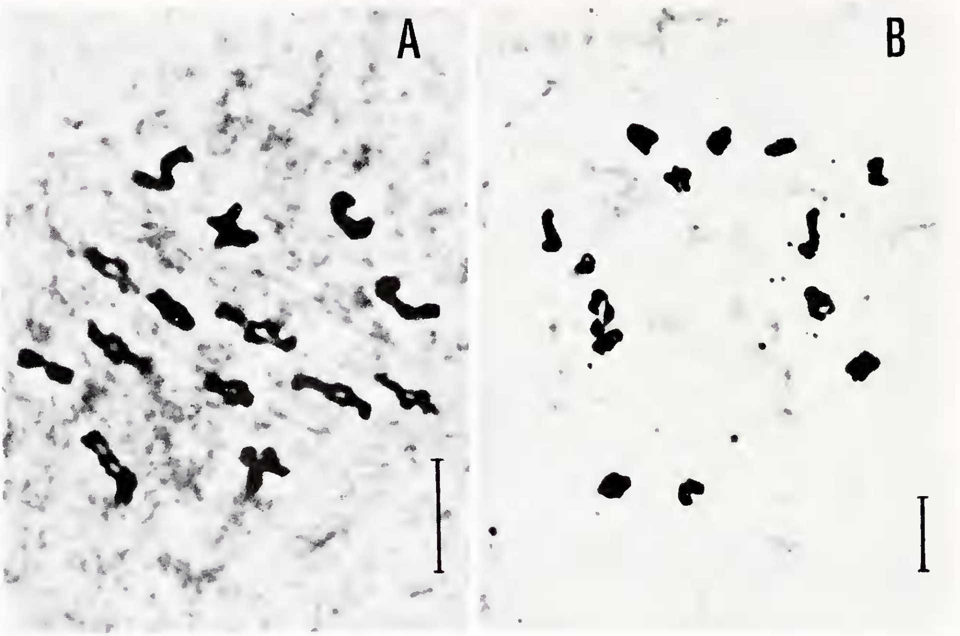

FIGURE 1. Meiotic chromosomes of *Isognomon alatus:* A, fourteen metaphase I chromosomes; B, fourteen metaphase II chromosomes. Scale bars represent 5 μ.

Chromosomes were examined with a 100X Zeiss phase-contrast objective, and suitable cells were photographed. Drawings were made to supplement the photographs.

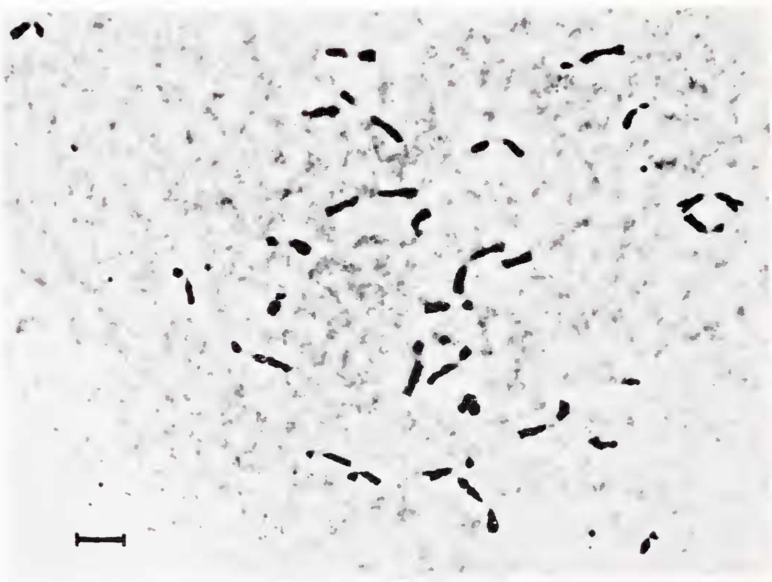

FIGURE 2. Twenty-eight colchicine-metaphase chromosomes of first cleavage in *Isognomon alatus.* Scale bar represents 5 μ.

Observations and Results

Flat tree oyster, I. alatus

Stripped and unfertilized eggs were oocytes at meiotic prophase I about diakinesis. After insemination, eggs proceeded to complete meiosis. Clear counts of 14 pairs of chromosomes were observed in 10 eggs at metaphase I (Fig. 1A) and 14 chromosomes in 14 eggs at metaphase II (Fig. 1B). The chromosome pairs are all homomorphic and isopyknotic with no evidence of specialized sex chromosomes. Chiasmata are present, indicating the occurrence of crossing-over of the genes. The diploid number of 28 was observed in well-spread cleavage metaphases or anaphases from both 30 embryos treated with colchicine and from 20 untreated embryos. Figure 2 shows a well-spread metaphase plate of a colchicine-treated embryo.

Five sets of 28 chromosomes in colchicine metaphase were tentatively arranged into pairs according to decreasing size. Total measured length of these chromosomes, arm ratio and general morphological appearance, as measured and observed in the photographic prints, were the criteria used for pairing. Using the mean and standard deviation of the 5 tentative arrangements in Figure 3, mean length extended from 6.0 ± 0.78 to 2.2 ± 0.03 μ or from 5.6 ± 0.25 to $2.1 \pm 0.07\%$ of the total length of the diploid complement. Arm ratios measured from 1.2 ± 0.01 to 2.6 ± 0.24. Five chromosome pairs may be tentatively classified as metacentrics and the others as submetacentrics (Levan, Fredga and Sandberg, 1964). Both metacentrics and submetacentrics are included among the longer and the shorter chromosomes. Apparent variation in chromosome size is probably due to nonsynchronous coiling of the chromosomes, both natural and colchicine-induced (see Fig. 3).

Atlantic pearl oyster, P. imbricata

Spawned and unfertilized eggs were at diakinesis of metaphase I. Fourteen homomorphic and isopyknotic chromosomes were counted in about 200 squashes of eggs at metaphase I (Fig. 4A). Chromosomes complete meiosis after insemination, and 14 chromosomes were observed in 24 eggs at metaphase II (Fig. 4B).

Twenty-eight chromosomes were counted in well-spread metaphase or anaphase plates in 8 first cleavage embryos untreated with colchicine. Figure 5 shows metaphase plates of first cleavages.

The chromosomes in drawings of two mitotic metaphase plates were tentatively arranged into 14 pairs in the same manner as done for *I. alatus* (Fig. 6). Mean length ranged from 7.8 ± 0.99 to 1.7 ± 0.44 μ (mean and standard deviation of two tentative arrangements in Fig. 6). Three or four pairs can be classified tentatively as subtelocentrics and the others as submeta- or metacentrics. These are, however, only preliminary observations, and there are very sizeable morphological variations of the chromosomes in the arrangement.

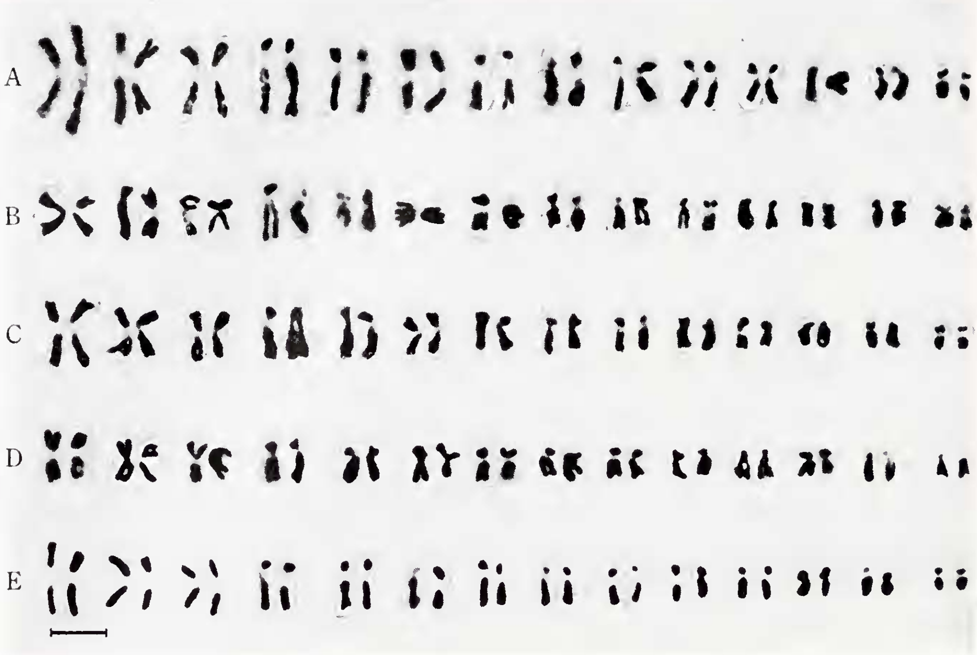

FIGURE 3. Five tentative arrangements of colchicine-metaphase chromosomes of early cleavages in *Isognomon alatus*. Scale bar represents 5 μ.

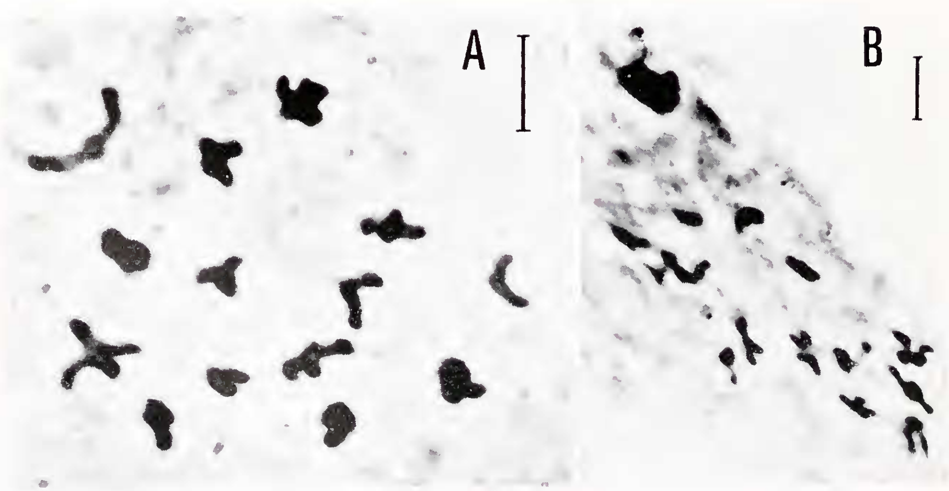

FIGURE 4. Meiotic chromosomes of *Pinctada imbricata*: A, fourteen metaphase I chromosomes; B, fourteen metaphase II chromosomes and first polar body. Scale bars represent 5 μ.

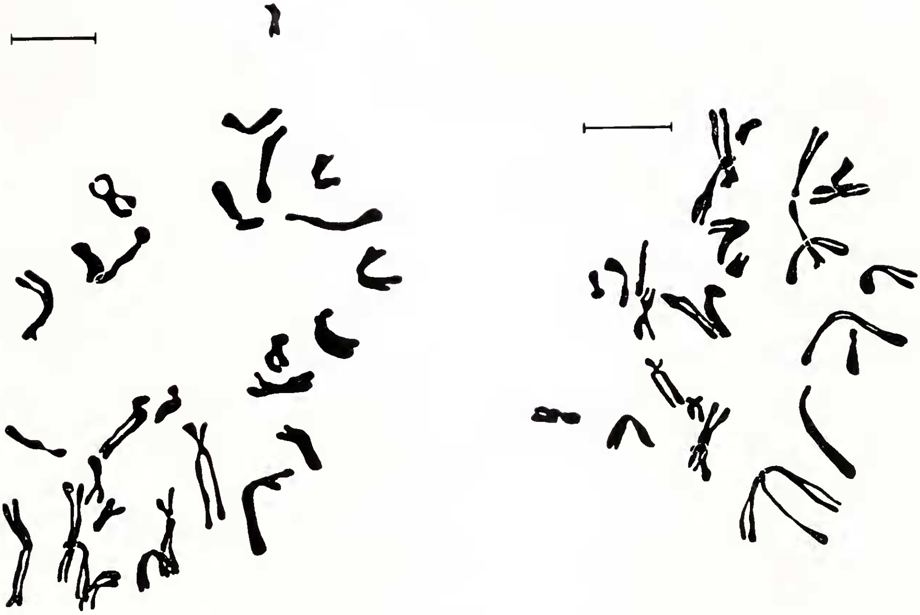

FIGURE 5. Two drawings of mitotic metaphase plates with twenty-eight chromosomes of first cleavages in *Pinctada imbricata*. Scale bars represent 5 μ.

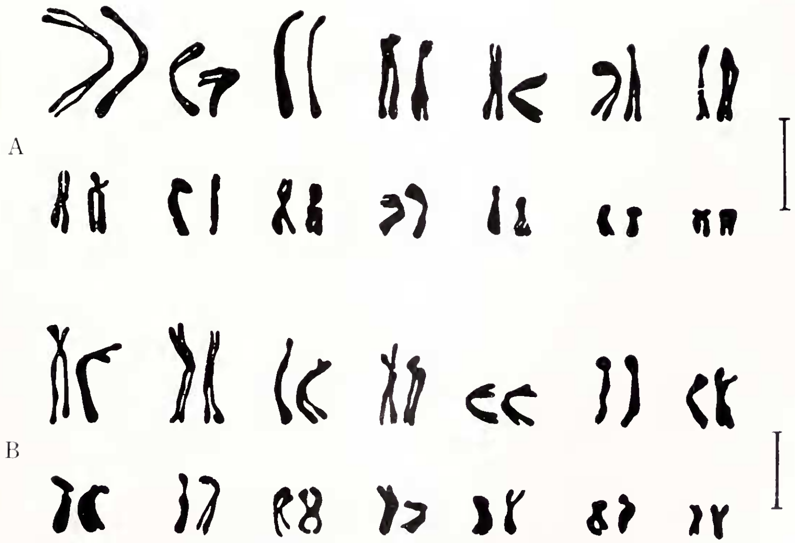

FIGURE 6. Two tentative arrangements of mitotic metaphase chromosomes of *Pinctada imbricata* from the drawings of Figure 5. Scale bars represent 5 μ.

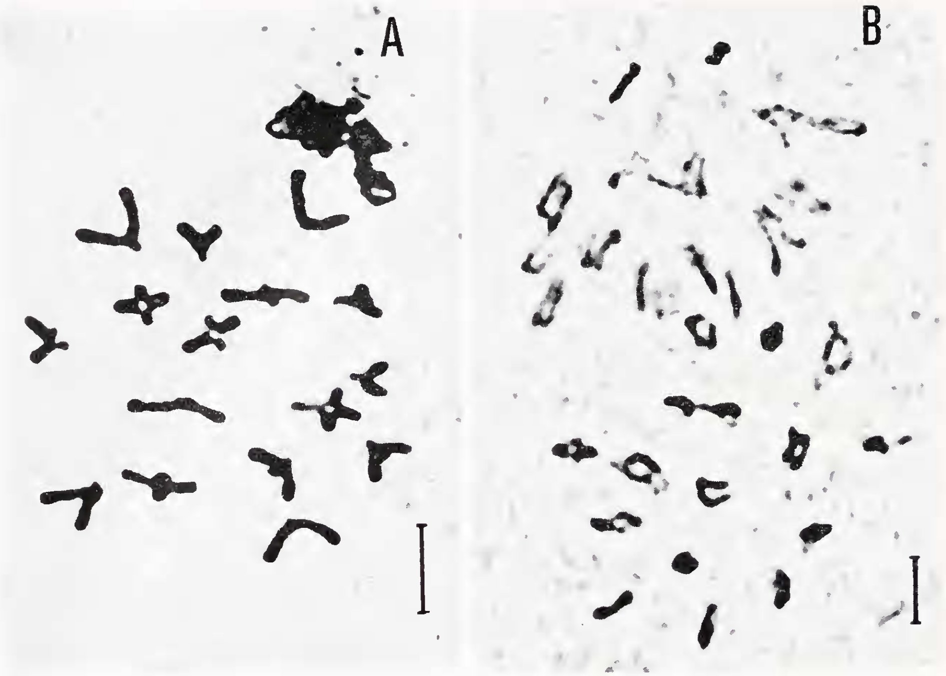

FIGURE 7. Meiotic chromosomes of *Argopecten irradians irradians:* A, sixteen metaphase I chromosomes; B, sixteen pairs of anaphase I chromosomes. Scale bars represent 5 μ.

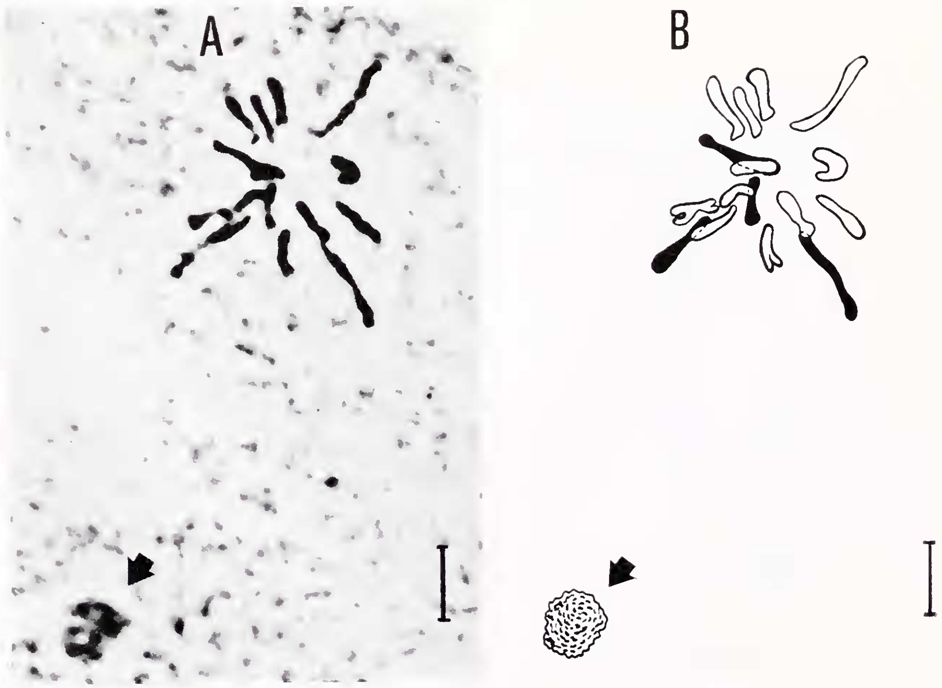

FIGURE 8. Meiotic chromosomes of *Argopecten irradians irradians:* A, sixteen metaphase II chromosomes and sperm nucleus (at arrow); B, drawing of the chromosomes shown in A.

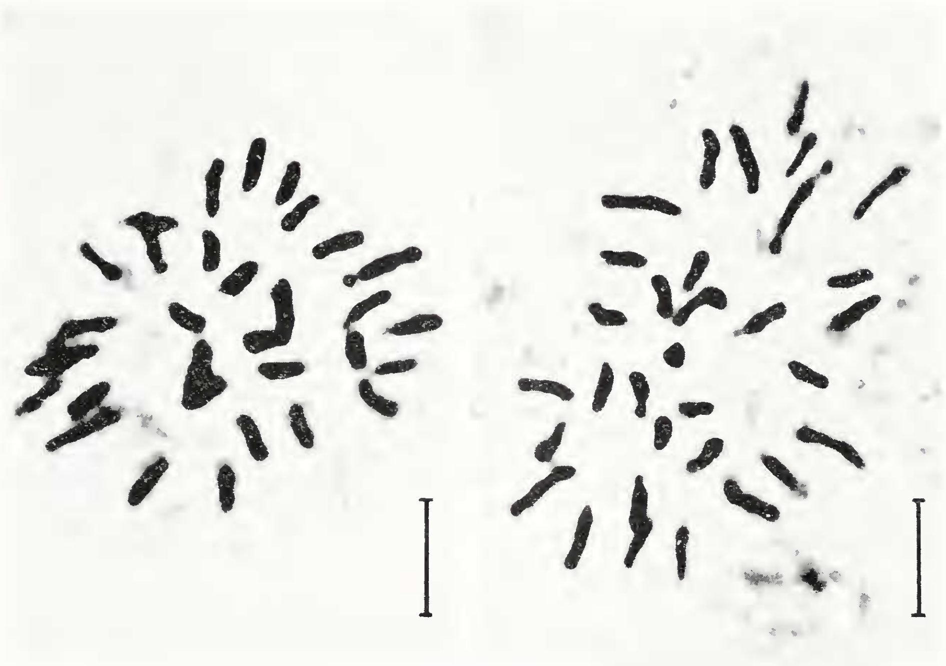

FIGURE 9. Thirty-two colchicine-metaphase chromosomes of first cleavages in *Argopecten irradians irradians*. Scale bar represents 5 μ.

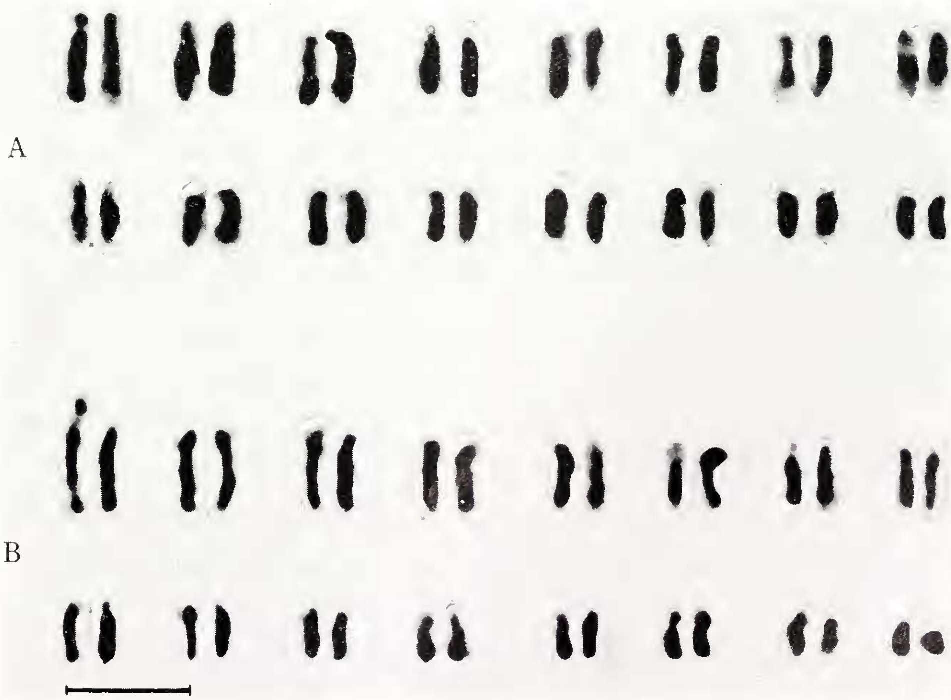

FIGURE 10. Two tentative arrangements of colchicine-metaphase chromosomes of first cleavages in *Argopecten irradians irradians*. Scale bar represents 5 μ.

Bay scallop, A. irradians irradians

Unfertilized eggs were at diakinesis or metaphase I. Sixteen homomorphic, isopyknotic chromosome pairs were observed in over 50 squashes of meiosis I at metaphase (Fig. 7A). (Figure 7B shows these chromosomes at anaphase I.) After insemination 16 chromosomes were counted in 6 eggs at metaphase II (Fig. 8).

Thirty-two chromosomes were counted in well-spread metaphase plates in 30 embryos treated with colchicine and in 20 untreated ones. Figure 9 shows colchicine metaphase plates of first cleavages.

The chromosomes in Figure 9 were tentatively arranged into 16 pairs (Fig. 10). It was difficult to recognize the centromere in some chromosomes, and there were large morphological variations because of the technique used. Even so, the chromosome complements seemed to consist of meta-, submeta-, subtelo- and telocentrics. Mean length ranged from 5.0 ± 1.67 to 1.8 ± 0.23 μ or from 5.2 ± 0.26 to $1.9 \pm 0.30\%$ of the total length of the diploid complement (mean and standard deviation of four tentative arrangements). The construction in one of the longest chromosomes might have been a technical artifact.

Discussion

There has been no report to date on the chromosome number of any species in the family Isognomonidae of the order Pterioida. The chromosome number ($2n = 28$) of *I. alatus* given above coincides with those of *Pinctada fucata* (Wada, 1976) and *P. imbricata* also given above. All of these species are in the superfamily Pteriacea, and are the only ones examined in this superfamily (Table I). Constancy of chromosome number here seems to be one additional example of the tendency for chromosome number to be stable within the family or superfamily of Pelecypoda (Menzel, 1968; Patterson, 1970; Ieyama and Inaba, 1974).

In the Japanese pearl oyster, *P. fucata,* about four of 14 pairs in the tentative arrangement of early cleavage metaphase chromosomes were identified as subtelocentrics and others as meta- or submetacentrics (Wada, 1976). In the study of the Atlantic pearl oyster reported here, three or four subtelocentrics were likewise observed, and the others were similarly meta- or submetacentrics. No one has previously compared the chromosomes of the Atlantic and the Japanese pearl oysters. In his taxonomic revision of the genus Pinctada, Ranson (1961) classified the Atlantic pearl oyster, which also has distribution in the Pacific Ocean, as well as the Indian Ocean, Persian Sea and Mediterranean Sea, as *Pinctada radiata* (Leach). He classified the Japanese pearl oyster, *P. martensii* (Dunker), separately from *P. radiata.* Recently, Kuroda, Habe and Oyama (1971) reported the Japanese oyster to be a subspecies, *P. fucata martensii* (Dunker), of the tropical Pacific oyster, *P. fucata* (Gould), which was reported to be synonymous with *P. radiata* by Hynd (1955). Hayes (1972) considered the Atlantic species to be a synonym of *P. imbricata* Röding. The similarity of chromosome number in the Japanese and Atlantic pearl oysters may have bearing on their taxonomy. It would be interesting to observe if chromosome behavior at meiosis in a hybrid of these two oysters appeared to be normal or abnormal. The latter would indicate chromosome dissimilarities in spite of karyotypic similarity.

TABLE I

Chromosome numbers of three families of pelecypod molluscs.

Species name	2n	n	Reference
Isognomonidae			
Isognomon alatus	28	14	Present study
Pteriidae			
Pinctada fucata	28	14	Wada, 1976
P. imbricata	28	14	Present study
Pectinidae			
Pecten maximus	38	19	Beaumont & Gruffydd, 1974
Chlamys varia	38	19	Beaumont & Gruffydd, 1974
Ch. distorta	38	19	Beaumont & Gruffydd, 1974
Ch. islandica	38	19	Beaumont & Gruffydd, 1974
Ch. opercularis	26	13	Beaumont & Gruffydd, 1974
Argopecten irradians irradians	32	16	Present study
Placopecten magellanicus	38	19	Beaumont & Gruffydd, 1974

In the family Pectinidae, the following chromosome numbers of six species were reported (Beaumont and Gruffydd, 1974): 2n = 38 for *Pecten maximus, Placopecten magellanicus, Chlamys varia, Ch. distorta* and *Ch. islandica* and 2n = 26 for *Chlamys opercularis.* The number (2n = 32) for *A. i. irradians* examined in the present study is different. Beaumont and Gruffydd (1974) implied that the mode of diploid number for the family Pectinidae was 2n = 38. They considered the possibility that the number of *Ch. opercularis* (2n = 26) was probably the result of "Robertsonian" centric fusion and that this species derived from an ancestral type with more than 26 (2n) chromosomes. At any rate, there seem to be some variations in chromosome number among species and genera within the family Pectinidae. This is in contrast with the tendency of chromosome numbers within other families of Bivalvia to be the same, as noted above. However, to confirm the seeming chromosome variation, further studies are needed on other species of this large family Pectinidae, which includes about 350 species (Hertlein, 1969).

A. i. irradians is hermaphroditic and the absence of defined sex chromosomes is expected. Self-fertilized and cross-fertilized embryos alike appear to develop with equal success in the laboratory, although self-fertilization is uncommon in nature (Castagna and Duggan, 1971; Castagna, 1975). No difference in incidence of chromosome irregularities in self- and cross-fertilized embryos was apparent on cursory examinations. More study would be needed, though, to confirm this impression.

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Summary

Chromosome number and morphology were observed in the squashed eggs and embryos of three bivalve species, *Isognomon alatus*, *Pinctada imbricata* and *Argopecten irradians irradians*. *I. alatus* has 14 pairs of chromosomes, all of which are either meta- or submetacentrics. Three or four of 14 chromosomal pairs of *P. imbricata* are subtelocentrics and the rest are submeta- or metacentrics. The chromosome complement of *P. imbricata* seems to be similar to that of *P. fucata*. Chromosome numbers of *A. i. irradians* are 16 (n) and 32 (2n) and are different from those of six Pectinidae species previously reported. The 16 pairs of chromosomes of *A. i. irradians* appear to consist of meta-, submeta-, subtelo-, and telocentrics.

LITERATURE CITED

Ahmed, M., and A. K. Sparks, 1967. A preliminary study of chromosomes of two species of oysters (*Ostrea lurida* and *Crassostrea gigas*). *J. Fish. Res. Board Can.*, **24**: 2155–2159.

Ahmed, M., and A. K. Sparks, 1970. Chromosome number, structure and autosomal polymorphisms in the marine mussels *Mytilus edulis* and *M. californianus*. *Biol. Bull.*, **138**: 1–13.

Beaumont, A. R., and Ll. D. Gruffydd, 1974. Studies on the chromosomes of the scallop *Pecten maximus* (L.) and related species. *J. Mar. Biol. Assoc. U.K.*, **54**: 713–718.

Castagna, M., 1975. Culture of the bay scallop, *Argopecten irradians*, in Virginia. *Mar. Fish. Rev.*, **37**: 19–24.

Castagna, M., and W. Duggan, 1971. Rearing the bay scallop, *Aequipecten irradians*. *Proc. Nat. Shellfish. Assoc.*, **61**: 80–85.

Hayes, H. L., 1972. The recent Pteriidae (Mollusca) of the Western Atlantic and Eastern Pacific Oceans. *Ph.D. Thesis, George Washington University*, 202 pp.

Hertlein, L. G., 1969. Family Pectinidae. Pages 348–373 in R. C. Moore, Ed., *Treatise on invertebrate paleontology, Part N, 1 (Mollusca 6, Bivalvia)*. Geological Society of America, Inc., Boulder, Colorado.

Hynd, J. S., 1955. A revision of the Australian pearl-shells, genus Pinctada (Lamellibranchia). *Aust. J. Mar. Fresh. Res.*, **6**: 98–137.

Ieyama, H., and A. Inaba, 1974. Chromosome number of ten species in four families of Pteriomorphia (Bivalvia). *Jpn. J. Malacol. (Venus)*, **33**: 129–137.

Ieyama, H., 1977. Studies on the chromosomes of two species in Mytilidae (Pteriomorphia, Bivalvia). *Jpn. J. Malacol. (Venus)*, **36**: 25–28.

Kuroda, T., T. Habe, and K. Oyama, 1971. *The sea shells of Sagami Bay*. Maruzen Co., Tokyo, 500 pp.

Levan, A., K. Fredga, and A. A. Sandberg, 1964. Nomenclature for centromeric position on chromosomes. *Hereditas*, **52**: 201–220.

Longwell, A. C., S. S. Stiles, and D. G. Smith, 1967. Chromosome complement of the American oyster *Crassostrea virginica*, as seen in meiotic and cleaving eggs. *Can. J. Genet. Cytol.*, **9**: 845–856.

MENZEL, R. W., 1968. Chromosome number in nine families of Pelecypod molluscs. *Nautilus,* **82**: 45–58.

MENZEL, R. W., AND M. Y. MENZEL, 1965. Chromosomes of two species of quahog clams and their hybrids. *Biol. Bull.,* **129**: 181–188.

PATTERSON, C. M., 1970. Chromosomes of molluscs. *Proceedings of Symposium on Mollusca, Mar. Biol. Assoc. India, Symposium Series 3, Part II:* 635–686.

RANSON, G., 1961. Les espéces d'huitres perlieres du genre Pinctada. *Mem. Inst. R. Sci. Nat. Belg.,* **67**: 1–93.

WADA, K., 1976. Number and gross morphology of chromosomes in the pearl oyster, *Pinctada fucata,* collected from two regions of Japan. *Jpn. J. Malacol. (Venus),* **35**: 9–14.